RESEARCH ON THE APPRECIATION OF THE HYGIENIC STATUS OF THE RAW MILK USING THE DOSAGE OF THE CATALASE ACTIVITY

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Abstract

Research conducted in recent years on the hygienic quality of milk, using the main indicators determined the existence of quality grids that were used and in the present paper helping to classify milk samples to different quality classes. Through this paper we tried to use the main rapid enzymatic tests to assess the freshness and hygiene of the raw material milk, depending on its retention time and any contamination with alterative microflora at the shelter / milking level, 25 samples of fresh milk, stored for 5 days under refrigeration conditions and experimentally contaminated milk were obtained by haemorrhage.

The determinations consisted in highlighting the acidity (°T), carrying out enzymatic tests catalase and reductase, identifying the number of somatic cells and the total number of germs.

Regarding the catalase test, it revealed a reduced microbial load in the fresh milk, indicating that it can be processed industrially or consumed after thermal processing. The milk refrigerated for 5 days and the experimentally contaminated milk presented medium and high microbial contamination, according to the catalase test, being risky, respectively unfit for consumption.

Regarding the test of reductase, it included the samples of fresh milk in class I of quality (lack of risks, immediate use after thermal processing); those of refrigerated milk in the second class of quality (exclusive use after industrial processing); those contaminated experimentally in quality class III (confiscation and prohibited use for food purposes).

As a final conclusion, he stated that enzymatic tests to identify catalase and reductase can be successfully used to determine the degree of contamination of milk and to determine its hygienic quality, the results of which are correlated with the total number of germs and with the titrable acidity.

Key words: milk, quality, enzymatic test

INTRODUCTION

Milk is a yellowish-white liquid that is secreted by the mammalian gland of mammals. [11], [12], [17]. From a physical point of view, milk is a complex system, being able to be considered an emulsion of type U / A, in which U represents the fatty phase formed by fat globules, and A the aqueous phase containing substances in colloidal form (proteins) or under dissolved form (lactose, mineral salts, water-soluble vitamins) [2], [5].

Milk is a basic element for all age groups, but also a raw material for a diverse range of products, both for food and industrial use [3], [10], [17].

The dairy industry in our country is in full development, the current phase being characterized by construction of new factories, re-equipping old factories, increasing production capacity and introducing modern technological processes [1], [9], [15].

Milk is one of the most commonly used foods in the daily menu of people and especially children, due to the nutritional processes it has. If we talk about the quality of milk, we are talking about its organoleptic properties, about the microbial load and last but not least about its physico-chemical properties [4], [8], [13], [16].

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The hygienic quality of the milk brings together all the sanitation criteria required by consumers and processors. This is the expression of the hygienic and toxicological stasis of milk (microbial load, number of somatic cells, impurities and content in residual, inhibitory and harmful substances) [6], [16].

The hygienic quality of milk produced strategically and indispensable for humans is a requirement of food security. It is required that the milk does not contain in dangerous doses microorganisms, somatic cells, undesirable substances, respectively harmful to the human body [7], [13].

After joining the European Union, Romania had to change the standards for milk quality, so that they comply with the standards imposed by the EU. The quality of milk is determined taking into account its chemical composition and the total number of germs (TGC), which should comply with the quality standards imposed on the market by European Union rules. Cow's milk is indispensable for human nutrition. The nutritional value of milk is determined by its balanced chemical composition, rich in fats, proteins, carbohydrates, mineral salts but also by its hygienic condition. The economic efficiency of cow farms is also influenced by the nutritious food and the hygienic qualities of the milk [5], [13].

Consumers on the Romanian market are increasingly informed and stricter regarding the quality of milk and milk products and whether they are obtained in accordance with quality and hygiene regulations. Some of the documents that require compliance with the rules of hygiene and quality of milk are the following: Reg CE 1881/2006 provides information on organochlorine and organophosphorus pesticides, Reg CE 37/2010 requires the absence of antibiotics in milk raw material, Reg CE no. 853/2004 provides information about the microbiological properties, respectively NTG / ml, max and NCS.ml, max. is 100,000, listeria 400,000, and cytogenes / 25ml must be absent according to SR EN ISO 11290-1.2. [9], [13].

Factors that influence the appearance of microorganisms in milk are: bactericidal or bacteriostatic power of milk, the presence in milk of growth factors of microorganisms, associations and antagonisms between microorganisms that contaminate milk, storage temperature of milk [8], [14].

The bactericidal or bacteriostatic power of milk is given by substances that inhibit the growth of microorganisms or destroy them, among them are: lactenins or agglutinins, lactate peroxidase and superoxide dismutase. Lactenins become active in fresh milk, because here there are no more anaerobic conditions to prevent their activity as in the udder. [9], [11], [14].

For these reasons, in this paper we have tried to use the main rapid enzymatic tests to assess the degree of freshness and hygiene of raw milk, depending on its shelf life and possible contamination with altered microflora at the shelter / room level.

MATERIAL AND METHODS

In the paper, the biological material used is represented by: fresh milk, refrigerated milk for 5 days and experimentally contaminated milk, its source being the Rediu farm, Iaşi.

Determination of the freshness of milk using acidity. Milk acidity can be considered one of the first indicators that can provide information about the freshness of milk and dairy products. Determination of milk acidity by titration can be done by several methods, such as: Thorner, Soxhlet-Henkel and Dornic. For the analyzes performed in this work, the Thorner method was used, a method in which the titration is performed with 0.1 n NaOH.

Enzymatic methods used to assess the freshness and hygienic quality of milk. The origin of the enzyme reductase is microbial, and its activity varies in direct proportion to the bacterial load of milk.

The essential property of this enzyme, on which the determination itself is based, is to reduce some colored solutions to the level of leukoderivatives and can be achieved with methylene blue or resazurin.

Assessing the hygiene status of raw milk by determining the number of somatic cells. This quantitative method has as a principle the counting of somatic cells in

milk, pigmented especially on a smear made, starting with a known volume of milk. EkoScope was used to determine them - it is a complete system used for counting somatic milk cells, produced by EON Trading, Bulgaria

Assessment of the hygiene of milk raw material by determining the total number of germs. The laser-scattering method was used to identify and quantify the total number of germs in milk. Bacterioscan 216R equipment was used after pre-preparation of the sample according to the method.

RESULTS AND DISCUSSIONS

The results obtained from the analysis of milk acidity. The three times of milk, fresh, refrigerated and experimentally contaminated were subjected to the acidity test, the results obtained from the analyzes being centralized in table 1.

Table 1 Milk acidity

Specification	Fresh milk	Chiles milk 5 days	Experimental contaminated milk
Average ($\bar{x}$)	17.55	20.03	21.39
Standard average error($\pm S\bar{x}$)	0.06	0.14	0.18
Coefficient of variation(v%)	1.59	3.47	4.21
Alternativ (S^2)	0.08	0.48	0.81
Standard deviation (s)	0.28	0.69	11.96

In the case of fresh milk, the average value is 17.55ºT, in the range of 15-19ºT, which demonstrates an acidity within normal limits.

In the case of refrigerated milk for 5 days, the average of the values of the results of the analyzes performed slightly exceeds the limits, being 20.03ºT. In some exceptional cases, the upper limit of 21ºT is also allowed, so, considering the time elapsed after milking, we can conclude that the refrigerated milk has an acidity that slightly exceeds the normal limits.

For experimentally contaminated milk, the results are different. The analytical value of the results is 21.39ºT, a value that determines a milk unsuitable for industrial processing.

Assessment of the hygienic quality of milk using the catalase test. During the analyzes, the catalase test was performed, its amount varying depending on the microbial load of the milk, which helped to establish the hygienic condition of the milk samples.

The values obtained from the analyzes performed on fresh milk show that its hygienic condition complies with the standards, which fall within the range of 0.1-1 cm^3 O_2 released, more precisely having an average of 0.77 ± 0.02 cm^3 O_2 (table 2).

Table 2 Establishing the hygienic quality of milk samples by catalase test

Specification	Fresh milk	Chiles milk 5 days	Experimental contaminated milk
Average ($\bar{x}$)	0.77	1.25	1.82
Standard average error($\pm S\bar{x}$)	0.02	0.04	0.06
Coefficient of variation(v%)	14.73	14.47	15.87
Alternativ (S^2)	0.01	0.03	0.08
Standard deviation (s)	0.11	0.18	0.29

In the case of refrigerated milk for 5 days, the situation is different, here the values obtained from the analyzes show a slightly non-compliant milk, the average analysis being 1.25 ± 0.04 cm^3 O_2, a value that does not fall within the limits imposed by the standard.

In the third case, the average value of experimentally contaminated milk (1.82 ± 0.06 cm^3 O_2) far exceeds the range of 0.1-1 cm^3 O_2 released, which demonstrates poor hygiene and a high degree of microbiological

contamination of milk. Highlighting the hygiene of milk by reductase test - methylene blue method.

The milk samples were subjected to the reductase test, a method that follows the time elapsed until the discoloration of the samples, which were thermo stated at a temperature of 37ºC and tinted with an aqueous solution of methylene blue. The values revealing the time elapsed until discoloration were highlighted in table 3.

In turn, for fresh milk, refrigerated for 5 days and for experimentally contaminated milk, analyzes were performed showing the quality class to which each type of milk belongs and its stability.

Thus, for fresh milk, the time of discoloration of the samples was between 221-259, with an average of 242.28′ and a variation of 4.94%, a value that falls within the limits imposed by standard 240 -300′ and is associated with or corresponding to quality class I (class which is defined by a sample discoloration time of 240′).

Table 3 Assessing the degree of hygiene of milk raw material, by identifying reductase with methylene blue

Specification	Fresh milk	Chiles milk 5 days	Experimental contaminated milk
Average ($\bar{x}$)	242.28	190.72	57.40
Standard average error ($\pm S\bar{x}$)	2.39	1.30	1.95
Coefficient of variation (v%)	4.94	3.41	16.94
Alternativ (S^2)	143.04	42.38	94.58
Standard deviation (s)	11.96	6.51	9.73

In the case of milk refrigerated for 5 days, the methylene blue reductase sample led to the following conclusions: the samples analyzed fall into quality categories I and II, mainly towards quality II (the time required until discoloration for quality I is 240′ , respectively 120′ for quality II), and for the analyzes performed within the project the time elapsed until discoloration was 190, 72′ with a coefficient of variation of 3.41%.

For the experimentally contaminated milk the situation of the results of the analyzes performed is slightly different: according to the average value of 57.40′, the samples of experimental contaminated milk belong to quality class III (with a time required until discoloration of 60′) being considered a poor quality product. The lowest value obtained in the analyzes is 28′ close to quality class IV, and the highest is 69′, with an average of 56.40′ and a coefficient of variation of 16.94%.

Assessment of the hygienic quality of the milk and the state of freshness by determining the number of somatic cells.

Determining the number of somatic cells in milk helps to establish the freshness of milk, the health of the mammary gland, representing an important indicator of its hygienic quality. In the present paper, the number of somatic cells on the three types of milk was determined, the results obtained being systematized in table 4.

Table 4 Assessing the degree of hygiene of raw milk, by identifying the number of somatic cells present

Specification	Fresh milk	Chiles milk 5 days	Experimental contaminated milk
Average ($\bar{x}$)	48562.00	53642.00	49700.00
Standard average error($\pm S\bar{x}$)	1400.30	1989.39	1557.65
Coefficient of variation(v%)	14.42	18.54	15.67
Alternativ (S^2)	49020683.33	98941600.00	60656666.67
Standard deviation (s)	7001.48	9946.94	7788.24

In the case of fresh milk, an average number of cells is found following the analysis of the 25 samples of 48562.00 cells / ml value lower than the standard value accepted by the European Union of 4000,000 cells / ml., Which helps to conclude that the milk analyzed is of superior hygienic quality, recommended for human consumption.

For refrigerated milk for 5 days the situation is slightly similar to that corresponding to fresh milk, the highest value obtained in the analyzes being 69000 cells / ml., And the lowest of 40,000 cells / ml with an average of 53642.00 cells/ml having a deviation of 18.54%. The graphic situation presented leads to the conclusion that refrigerated milk for 5 days contains a number of somatic cells below 400000 cells/ml., In line with EU quality standards, representing a raw material compliant for the realization of other finished products for human consumption.

In the case of experimentally contaminated milk, the average value of the results of the analyzes performed for each milk sample is 49700.00 cells / ml., With a coefficient of variation of 15.67%. value lower than the standard value accepted by the European Union of 4000,000 cells / ml.

Assessment of the hygienic quality of the milk and the state of freshness by determining the total number of germs. Determining the total number of germs provides information about the hygiene of milking and utensils used for milking, but also on the storage conditions of milk immediately after milking, is an important indicator of the hygienic quality of milk.

In the samples of fresh milk, following the analyzes performed, an average analytical value of 9056.00 germs / ml was determined. (fig. 1), value that falls within the limit imposed by European Union standards, limit of 100,000 germs / ml. This value demonstrates that the milk that was subjected to the analysis is a milk that was harvested in appropriate hygienic conditions for milking, with a low microbial load which makes it a milk for human consumption.

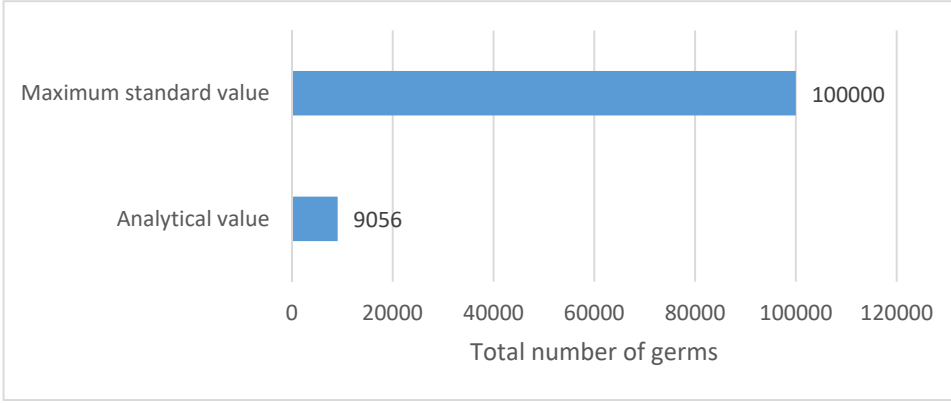

Fig. 1 Results obtained by determining the total number of germs for fresh mil

In the case of milk refrigerated for 5 days, the average of the test results which were analyzed is 24520.00 germs / ml (fig. 2), with a coefficient of variation of 7.08%, the highest value being 28000 germs / ml, and the lowest of 22,200 germs / ml.

The obtained results show that milk was harvested in optimal conditions of hygiene and that it was kept in appropriate environmental conditions, without having a high microbial load and thus being optimal for its processing and industrialization.

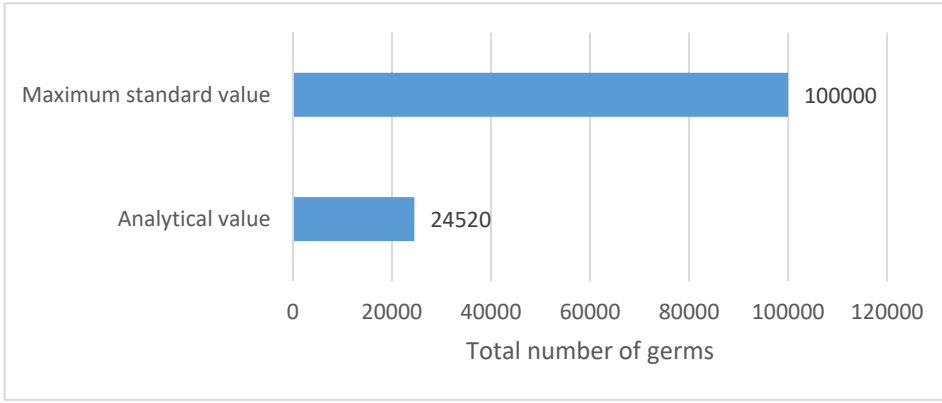

Fig. 2 Results obtined after determining the total number of germs for refrigerated milk for 5 days

For experimentally contaminated milk, the situation of the results of the analyzes performed, presented graphically, is different. In this case, the average analytical value is 107840.08 germs.ml. (fig. 3), a value much higher than the limit imposed by EU standards, the limit of 100000 germs / ml., Which proves a high microbial load of said milk analyzes, which makes it milk unfit for industrial processing, respectively for human consumption, being harvested and stored in poor hygiene conditions. The highest value recorded here is 135,000 germs / ml.

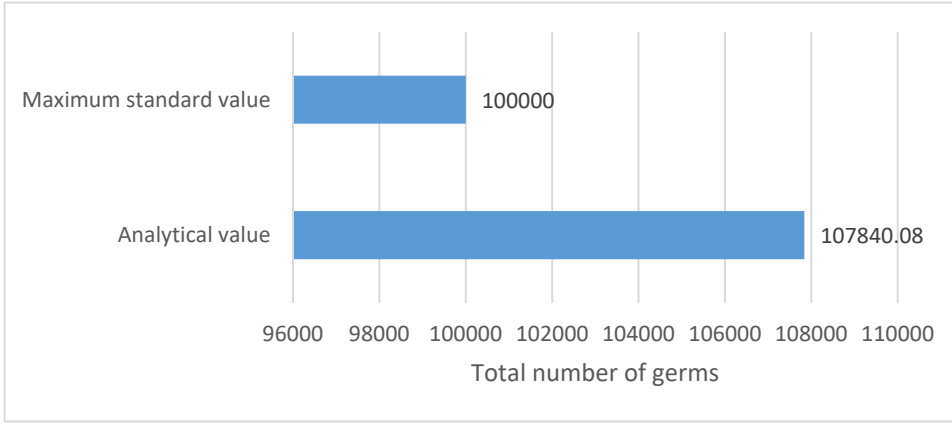

Fig. 3 Results obtined after determining the total number of germs for experimentally contaminated milk

CONCLUSION

Following our own research and analysis of enzymatic analysis methods, the following conclusions were drawn:

- the degree of freshness of the milk, assessed by the titratable acidity values, complied with the standard for fresh milk (15-19ºT) and in the case of milk refrigerated for 5 days and experimentally contaminated milk, the values of the analytical average exceeded the standard range, making a milk unfit for human consumption;

- the catalase test revealed a low microbial load in fresh milk, indicating that it can be processed industrially or consumed after heat processing. The milk refrigerated for 5 days and the experimentally contaminated one showed medium and high microbial contamination, according to the catalase test, being risky, respectively unfit for consumption

- the reductase test included the samples of fresh milk in quality class I (no risks, use immediately after thermal processing); those of refrigerated milk in the second quality class (exclusive use after industrial processing); those experimentally contaminated in quality class III (confiscation and use prohibited for food purposes).

- the analysis for somatic cell content indicated, for all three categories of milk studied, average values, below the legal limit of 400000 cells / ml., even for samples of artificially refrigerated and contaminated milk, emphasizing that dairy cows do not have problems with the health of the udder and that the results of enzymatic tests and acidity are influenced by the dynamics of the microbial load under the influence of storage time or contamination after milking.

-for the microbial load, in the case of fresh milk (9050 germs / ml) and refrigerated milk (24520 germs/ml) values were obtained that fall within the limit imposed by European Union standards (100,000 germs / ml). In the case of experimentally contaminated milk, the mean analytical value is 107840.08 germs / ml., Hygienically non-compliant.

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